

**The Annual Final Scientific Report:**

**Development of Convergence Nanoparticles for Multi-Modal  
Bio-Medical Imaging**

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Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE <b>18 SEP 2008</b>		2. REPORT TYPE <b>FInal</b>		3. DATES COVERED <b>01-02-2007 to 01-02-2008</b>	
4. TITLE AND SUBTITLE <b>Development of Convergence Nanoparticles for Multi-Modal Bio-Medical Imaging</b>				5a. CONTRACT NUMBER <b>FA48690714016</b>	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) <b>Jinwoo Cheon</b>				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Yonsei University,134 Shinchon-dong, Seodaemun-gu,Seoul 120-749,KR,120-749</b>				8. PERFORMING ORGANIZATION REPORT NUMBER <b>N/A</b>	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) <b>AOARD, UNIT 45002, APO, AP, 96337-5002</b>				10. SPONSOR/MONITOR'S ACRONYM(S) <b>AOARD</b>	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) <b>AOARD-074016</b>	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT <b>The purpose of this project is to develop a novel, highly versatile, multi-functional convergence nanoparticle system for their advanced biomedical applications. Inorganic nanoparticles are now emerging as a promising candidate to revolutionize current science and technology. By utilizing inorganic nanoparticles, it is possible to have the ability to control physical/chemical properties of materials as we desire, which is no allowed incurrent technology. Furthermore, these inorganic nanoparticles exhibit improved physical/chemical properties, compared to classical bulk materials. Such enhanced properties of inorganic nanoparticles make them useful as key components for futuristic nanodevice applications although there have been remarkable progresses in the synthesis of inorganic nanoparticles during the past decade, currently developed nanoparticles possess only simple and basic functions and therefore have difficulties to be utilized in advanced applications. To develop multi-functional convergence nanoparticles, we planed three-year research project. As the first stage, in this year, we fabricated prototype convergence nanoparticles and we first studied multi-functionality of magnetic nanoparticle, MR (magnetic resonance) contrast effect and heat generation ability. Based on the magnetic nanoparticle, we synthesized prototyped convergence nanoparticles by conjugating with optical component and radioisotope. Then, we have accomplished feasibility test on the biological system such as Amyloid &amp;#946; aggregation and lymph node detection.</b>					
15. SUBJECT TERMS <b>Bio-applications, Nanotechnology</b>					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>Same as Report (SAR)</b>	18. NUMBER OF PAGES <b>17</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

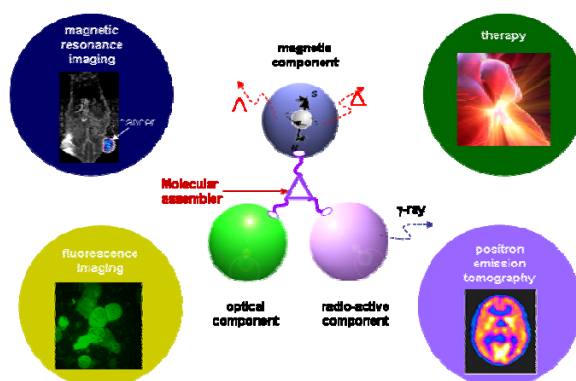
## **1. Abstract**

The purpose of this project is to develop a novel, highly versatile, multi-functional convergence nanoparticle system for their advanced biomedical applications. Inorganic nanoparticles are now emerging as a promising candidate to revolutionize current science and technology. By utilizing inorganic nanoparticles, it is possible to have the ability to control physical/chemical properties of materials as we desire, which is not allowed in current technology. Furthermore, these inorganic nanoparticles exhibit improved physical/chemical properties, compared to classical bulk materials. Such enhanced properties of inorganic nanoparticles make them useful as key components for futuristic nanodevice applications although there have been remarkable progresses in the synthesis of inorganic nanoparticles during the past decade, currently developed nanoparticles possess only simple and basic functions and therefore have difficulties to be utilized in advanced applications. To develop multi-functional convergence nanoparticles, we planned three-year research project. As the first stage, in this year, we fabricated prototype convergence nanoparticles and we first studied multi-functionality of magnetic nanoparticle, MR (magnetic resonance) contrast effect and heat generation ability. Based on the magnetic nanoparticle, we synthesized prototyped convergence nanoparticles by conjugating with optical component and radioisotope. Then, we have accomplished feasibility test on the biological system such as Amyloid  $\beta$  aggregation and lymph node detection.

## 2. Introduction

Inorganic nanoparticles with unique quantum mechanical properties have the potential to revolutionize current classical mechanics-based science and industry. By utilizing inorganic nanoparticles, it is possible to have the ability to control physical/chemical properties of materials as we desire, which is not allowed in current technology. Furthermore, these inorganic nanoparticles exhibit improved optical, magnetic, and electronic properties, compared to classical bulk materials. When these tiny materials get into biological system, their exceptionally enhanced properties arising from such nanoscale properties can enable them to be utilized as key probes and vectors for next-generation ultra-sensitive detection and highly efficient therapeutic systems. During past few years, researches on this field have been explosively carried out and some of successful studies have shown such possibilities in part. However, despite such remarkable progresses in bio-medical applications of inorganic nanoparticles, currently developed nanoparticles possess only simple and basic functions and therefore have difficulties to be utilized in advanced applications. For the successes in the next-generation nanotechnology-based bio-medical applications, it is necessary prerequisite to secure advanced inorganic nanoparticle systems.

For that reason, we proposed a novel, highly versatile, multi-functional convergence nanoparticle system for their advanced biomedical applications (**Scheme 1**). We fabricated prototype convergence nanoparticles by integrating individual nanoparticle components with its unique function into a single nanosystem.



**Scheme 1.** Convergence nanoparticle systems.

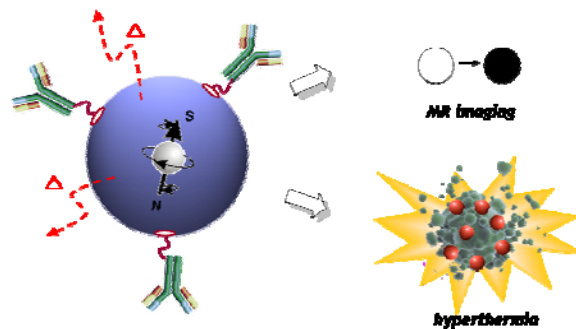
### 3. Approach

#### 1) Approach

##### A. Fabrication of magnetic nanoparticles for simultaneous diagnosis and hyperthermia treatments

Magnetic nanoparticles have novel magnetic properties arising from nanoscale phenomena which allow versatile application in biological fields. For example, magnetic nanoparticles have been widely used as a diagnostic agent for MRI. Besides their usage as a MRI contrast agent, magnetic nanoparticle can be also used as a heat generator for hyperthermia. Under alternating current (AC) magnetic field, the electron spins of magnetic nanoparticle are flipped along with the external magnetic field change. During this process, the energy difference between spin states generates heat which can deteriorate diseased tissues.

In order to fabricate high quality magnetic nanoparticle, we have been developed high temperature molecular precursor method. Among the various magnetic nanoparticles, we focused on fabrication of  $\text{MnFe}_2\text{O}_4$  which has superior magnetic property compared to other types of metal ferrites.



**Figure 1.** Magnetic nanoparticle for disease diagnosis and therapy.

##### B. Fabrication of a convergence nanoparticle system

Convergence nanoparticles can be fabricated through 1) self-assembly of nanoparticles using molecular assemblers or 2) selective secondary nucleation on top of the seed nanoparticles. Since the chemical/biological molecules can be designed and synthesized to have high symmetry and have the ability of self-recognition and assembly, the use of these molecular assemblers enables the systematic construction of multi-component convergence nanoparticles. In the case of the formation of convergence nanoparticles through secondary nucleation on the seed, modulation of similarity of the lattice geometry between seeds and secondary materials, affinity between two materials such as alloying, electro chemical potential differences, activity of a specific surface and catalytic activity is important.

### C. Multi-modal utilizations of convergence nanoparticles

The multi-component convergence nanoparticle system can enable multimodal multiplexing imaging and detection of biological processes by conjugating with bio-active materials. Conventional detection systems are operated independently and each detection system has own advantages and disadvantages. However, convergence nanoparticles can overcome the many shortcomings that are present for single imaging modality methods. As a model case study, we are planning to utilize magnetic-optical convergence nanoparticles for the dual-mode detection of proteins (amyloid  $\beta$  protein) or lymph node.

#### **2) Uniqueness of approach**

Although there have been remarkable progresses on the bio-medical applications of nanoparticles, currently developed techniques have been limited to mono-functional single component system and the realization of multi-modal biomedical applications of nanoparticles has been elusive. Convergence nanoparticle possesses i) multi-functionalities which enables accurate multi-modal diagnosis and pin-pointing therapy and have potential for ii) collective physical/chemical properties arising from synergistic communications between nanoparticle components and highly enhanced properties. With developed convergence nanoparticle system, it is possible to perform not only multi-mode detection system but also further applications such as sensing, information, navigation, and actuation in a single nanosystem. Therefore it can revolutionize current biomedical sciences and techniques including biosensor, cell trafficking, and cancer diagnosis.

#### **3) Research contents**

The 1st stage (2007.03-2008.02): Ability study of proto-type dual-mode convergence nanoparticles for their dual mode detection

- Fabrication of magnetic nanoparticles as a MRI contrast agent and heat generator
- Fabrication of proto-type convergence nanoparticle via combination of magnetic nanoparticle and fluorescent dye
- MRI and optical detection of amyloid  $\beta$  ( $A\beta$ ) protein by utilizing the developed convergence nanoparticles

The 2nd /3rd stage (2008-2009): Convergence nanoparticles for multi-modal bio-medical applications

## 4. Results and discussion

### 4-1. Fabrication of magnetic nanoparticles for simultaneous diagnosis and hyperthermia treatments

At the early stage of this project, we have investigated on the heat generation efficiency of magnetic nanoparticles, which make possible them to use for both cancer diagnosis and therapy. Among the various nanoparticles,  $\text{MnFe}_2\text{O}_4$  (MnMEIO; manganese doped magnetism engineered iron oxide), which previously showed excellent MR contrasting effect (*Nature Medicine* **2007**), was investigated.

$\text{MnFe}_2\text{O}_4$  are synthesized via high temperature decomposition of  $\text{MnCl}_2$  and  $\text{Fe}(\text{acac})_3$  ( $\text{acac} = 2,4\text{-pentadione}$ ) in the presence of oleylamine and oleic acid as capping molecules following known methods. Synthesized nanoparticles are 15 nm and high monodispersity . (Figure 2a)

Synthesized nanoparticles (1 mg/ml (Mn+Fe)) are mixed with cancer cell (MCF7) and heat generation efficacy was measured with the cell viability under the AC magnetic fields (500kHz). Live cells are stained with Calcein-acetoxymethyl(AM) Assay and shown as green color. As shown in figure 2, MnMEIO nanoparticle shows significant cancer cell killing effect with the AC magnetic field irradiation compared to the case without AC magnetic field.

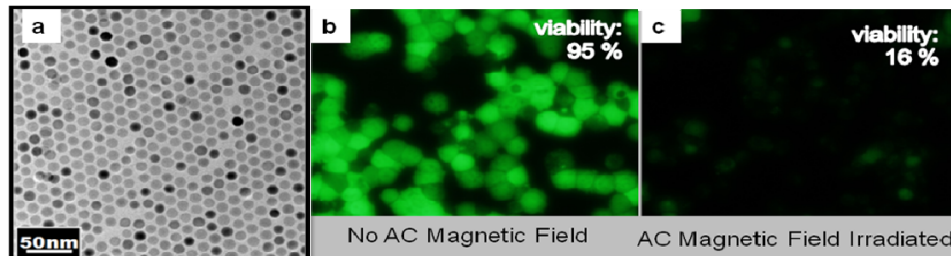


Figure 2. (a) TEM image of synthesized MnMEIO nanoparticles. When synthesized nanoparticles are mixed with cancer cells, compared to cancer cells without AC magnetic field (b), cancer cells which are applied AC magnetic Field shows significant

### 4-2 Fabrication of convergence nanoparticles and their utilization on the detection of the biological events.

#### 4-2-1 Magnetic nanoparticle for $\text{A}\beta$ aggregation detection

In next stage, we fabricate prototype convergence nanoparticles,  $\text{Co@Pt-Au}$ , through epitaxial growth on the seed.

First,  $\text{Co@Pt}$  was synthesized following the previously reported method. Then, Au is then deposited on the  $\text{Co@Pt}$  nanoparticles via hydrogenation of  $\text{AuCl}(\text{PPh}_3)$  by purging with a 4%  $\text{H}_2/\text{Ar}$  mixture. The  $\text{Co@Pt-Au}$  nanoparticles obtained in this

manner have a heterodimer structure composed of 6 nm Co@Pt and 9 nm Au. Synthesized nanoparticles show both superparamagnetic properties from Co@Pt and optical properties from gold component. (Figure 3)

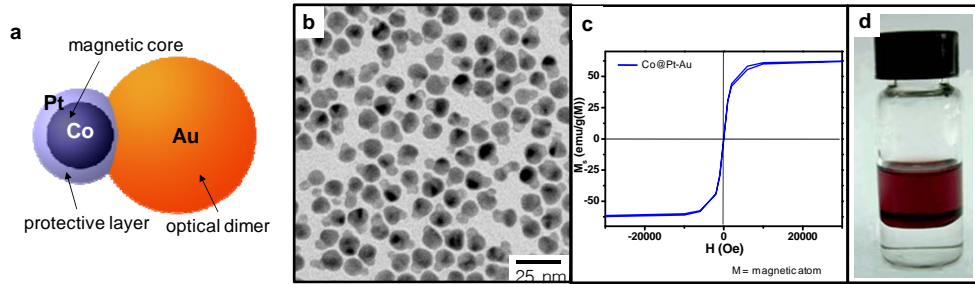


Figure 3. (a) Schematics and (b) TEM images of synthesized Co@Pt-Au. The synthesized nanoparticles show superparamagnetic properties (c) and have red color from Au component (d).

As a feasibility test, we examined A $\beta$  assembly detection. For that purpose, nanoparticles conjugated with neutravidins (NTV) which can successfully recognize biotin functionalized A $\beta$  assemblies. Because A $\beta$  peptide forms various assemblies from oligomer to protofibril and to fibril as their concentration increases, assembled nanoparticles due to A $\beta$  aggregates are expected to show increased signal compared to free nanoparticles. In the T2 image of Co@Pt-Au solution with various concentration of A $\beta_{40}$  peptides (0, 0.5, 1, 5, 10, 32, 80, 250  $\mu$ M), these samples showed that significant contrast changes take place from none to dark signal. The color coded images of T2 weighted signals more clearly depict the changes taking place as the A $\beta_{40}$  concentration increases. Especially, at the A $\beta$  concentration below 10  $\mu$ M, the sharp changes on  $\Delta$ T2 value is observed compared to solutions with A $\beta_{40}$  concentration upper than 10  $\mu$ M at which fibril forms. It demonstrates that MRI is a highly sensitive probe for detecting the early stages of A $\beta_{40}$  self-assembly (protofibril).

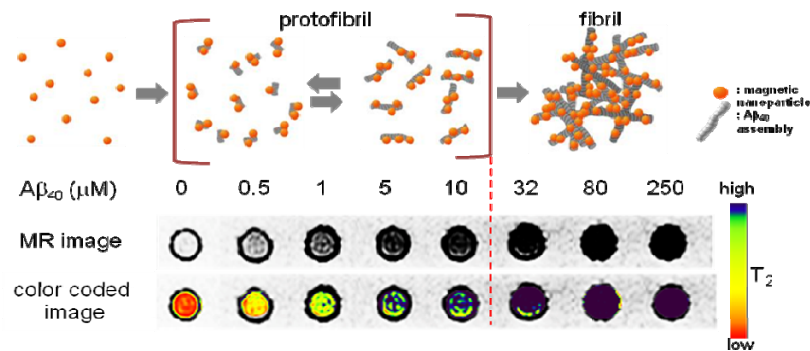


Figure 4. MR signal changes depending on degree of A $\beta$  assemblies. MR image



shows gradual changes from none to grey and to black and color coded image shows the signal changes more clearly.

#### 4-2-1 PET-MRI dual-modal nanoprobe for lymph nodes detection

By hybridization of magnetic nanoparticle, MnMEIO with radioisotope ( $^{124}\text{I}$ ), we could successfully perform the dual modal nanoprobe for PET and MRI imaging. Because PET radionuclide  $^{124}\text{I}$  can be directly conjugated to tyrosine in serum albumin (SA), SA coated MnMEIO is an efficient platform for formation of PET/MRI hybrid nanoprobe. (Figure 5a) Figure 5 depicts in vivo PET, MR, and PET/MR fusion images of SLN of Sprague-Dawley rats after  $^{124}\text{I}$ -SA-MnMEIO nanoprobe ( $320\text{ }\mu\text{g (Fe)/ kg}$ ,  $^{124}\text{I}$  activity:  $100\text{ }\mu\text{Ci}$ ) were injected to the right forepaw (injection site: I). In the coronal view of PET (Figure 5b), brachial lymph node, which is the first SLN, is clearly visualized as red hot spots after administration of  $^{124}\text{I}$ -SA-MnMEIO probes. Similarly, black spot is also observed by MR imaging (white arrow, Figure 5c) and the position of brachial lymph nodes is perfectly matched in PET/MR fusion image (figure 5d)

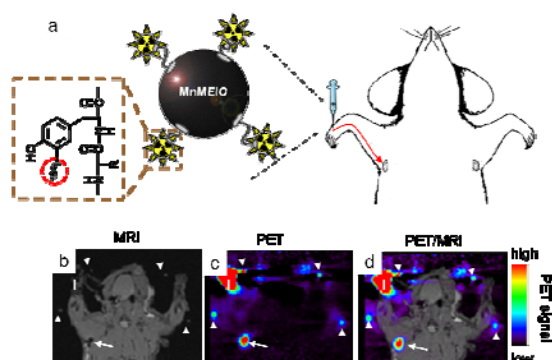


Figure 5. (a) Dual modal PET-MRI probe and schematics of lymph node imaging. (b) MRI, (c) PET, (d) PET-MRI fusion image of dual probe injected mouse. (I: injection site)

## **5. Pay-off**

This year project is for the demonstration of prototype convergence nanoparticles so these successful results can make possible to develop advanced convergence nanosystems for on going projects (2008~2009). As we described in previous section, convergence nanoparticle can be utilized for the multi-modal imaging because of their integrated properties, which can enhance the diagnostic accuracy and detection sensitivity. In addition, our convergence nanoparticle system also can be potentially further applied to have abilities such as sensing, information, navigation, and actuation in a single nanosystem and therefore can revolutionize current biomedical sciences and techniques including biosensor, cell trafficking, and cancer diagnosis. For example, convergence nanoparticle made by combination of magnetic nanoparticle mentioned in previous session, which has MR contrasting effect and heat generating ability, and fluorescent dye can be utilized as a simultaneous ultra sensitive diagnostic probe and disease treatment agent.

## 6. Summary

As a first stage in three year project for the development of convergence nanoparticles for multi-modal biological imaging, we successfully demonstrate the basic concepts for the formation of convergence nanoparticles and also find out their capability for the biological imaging. In this project, firstly, (1) we focused on the study of multi-functionality including MR contrasting ability and therapeutic ability of MnMEIO nanoparticles. (2) Then, based on the magnetic nanoparticles, we fabricate prototype convergence nanoparticles, Co@Pt-Au nanoparticles (epitaxial growth) and MnMEIO-radioisotope (hybridization using molecular linker). (3) With developed nanoparticles, we also perform the feasibility test for the detection of the biological event, A $\beta$  assemble and lymph node.

Based on these results, we will further investigate for the development of advanced convergence nanoparticles for the fault free diagnosis of disease.

## 7. References

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- J. Cheon *et al.* *J. Am. Chem. Soc.* **2005**, *127*, 9992.
- J. Cheon *et al.* *Nature Medine* **2007**, *13*, 95.

## 8. Research outputs

### 8-1. Publications

1. Cheon, J. *et al.*

“Nanoparticle assisted magnetic resonance imaging of the early-reversible stage of amyloid  $\beta$  self-assembly”

*Chem. Commun.* **2008**, 2197. [I.F. = 5.141]

2. Cheon, J. *et al.*

“A hybrid nanoparticle probe for dual-modality positron emission tomography and magnetic resonance imaging”

*Angew. Chem. Int. Ed.* **2008**, 47, 6259. [I.F. = 10.031]

### 8-2. Conferences

1. Cheon, J.

"Nanomaterials for Biomedical Diagnosis: Convergence Nano-structures and MEIO"

Designs for the Future: Promising Fields of Science and Technology, ICISTS-KAIST, Daejeon, Korea, July 24, 2007.

2. Cheon, J.

"Magnetic Nanoparticles for Bio-Medical Applications"

Materials Research Society Meeting, MRS, Boston, MA, U S A, Nov. 25, 2007.

3. Cheon, J.

"Fabrication of Hetero-Structured Co@Pt-Au Nanoparticles for the Biological Applications"

Materials Research Society Meeting, MRS, Boston, MA, U S A, Nov. 27, 2007

## 9. Financial Reports

I. Planned Expenditure (Nov. 1, 2004~ Oct. 31, 2005)	II. EXPENDITURES
<b>SALARIES</b> A. SENIOR PERSONNEL \$24,000 B. OTHER PERSONNEL \$ 12,000	<b>SALARIES</b> A. SENIOR PERSONNEL: \$ 24,000 Jinwoo Cheon : \$ 2,000 * 12 month = \$ 24,000 B. OTHER PERSONNEL \$ 12,000 Jae-Hyun Lee: \$ 1,000 * 6 = \$ 6,000 Jin-sil Choi: \$ 1,000 * 6 = \$ 6,000
D. EQUIPMENT \$ 0 E. TRAVEL \$ 3,000  F. TUITION REF 2.12.13 \$ 0	D. EQUIPMENT \$ 0 E. TRAVEL \$ 3,000 MRS, Boston Jin-sil Choi: Airfare: \$ 1,800 + Travel Cost: \$1,200 = \$ 3,000 F. TUITION REF 2.12.13 \$ 0
G. OTHER DIRECT COSTS \$ 56,200 1. SUPPLIES/MATERIALS \$ 13,250  2. COMM'S/SHIPPING \$ 1,000 3. OTHER \$ 3,000	G. OTHER DIRECT COSTS \$ 3,200 1. SUPPLIES/MATERIALS \$ 13,250 * See the detailed materials sheet. 2. Utility \$1,000 telecommunication \$50 * 12 = \$600 office supplies \$1400 copies \$1000
J. TOTAL DIRECT EXPENSES AND FACILITIES AND ADMINISTRATION EXPENSES Overhead \$18,750	J. TOTAL DIRECT EXPENSES AND FACILITIES AND ADMINISTRATION EXPENSES Overhead \$18,750
Total: \$75,000	Total: \$75,000

\* Detailed materials sheet

Item	Unit price (\$)	Amount	Total (\$)
Iron pentacarbonyl (Strem 26-2800, 250 g)	30.00	15	150.00
Dicobalt octacarbonyl (Fluka 60811, 25g )	200.00	10	2000.00
Platinum acetylacetonate (Aldrich 282782, 5g)	500.00	5	2500.00
Potassium tetrachloroaurate (Riedel 12526, 5g)	500.00	5	2500.00
Oleylamine (Aldrich O7805, 500g)	130.00	10	1300.00
Oleic acid (Sigma O1383, 25 g)	150.00	10	1500.00
Sephadex G-25(Sigma S5772, 25g)	180.00	5	900.00
TBE buffer (Sigma T4415, 10x, 20 L)	230.00	2	460.00
M <sub>2</sub> C <sub>2</sub> H	165.00	10	1650.00
1,2-Hexadecanediol, Tech (Aldrich 213748, 50g)	132.0	1	132.00
Iron(III) acetylacetonate (Aldrich 517003, 50g)	158.0	1	158.00
		3	
<b>Total</b>			<b>\$ 13,250</b>

## Nanoparticle assisted magnetic resonance imaging of the early reversible stages of amyloid $\beta$ self-assembly†

Jin-sil Choi,<sup>a</sup> Hyuck Jae Choi,<sup>b</sup> Dae Chul Jung,<sup>b</sup> Joo-Hyuk Lee<sup>b</sup> and Jinwoo Cheon<sup>a\*</sup>

Received (in Cambridge, UK) 26th February 2008, Accepted 19th March 2008

First published as an Advance Article on the web 11th April 2008

DOI: 10.1039/b803294g

Co@Pt-Au nanoparticles, which have enhanced magnetism and high stability in aqueous media, are utilized in conjunction with MRI to monitor the structural evolution of A $\beta$  assemblies, especially A $\beta$  protofibrils in the early reversible stages.

Self-assembly is a term used to describe the spontaneous and intermolecular force-driven organization of components into ordered structures. Processes of this type play crucial roles in cellular functions, and in the construction of higher level structures, such as lipid bilayers in cell membranes, double helical DNA and proteins in quaternary structures.<sup>1</sup> In particular, self-assembly of the amyloid  $\beta$  (A $\beta$ ) peptide, leading to malfunction of the neural system, is a known pathogenic process in Alzheimer's disease.<sup>2</sup> Recently, Au nanoparticles have been successfully employed for the highly sensitive detection of A $\beta$  assemblies in optical sensing or bio-bar code systems.<sup>3</sup>

In a magnetic resonance imaging (MRI)-based sensing system, advantage is taken of the fact that the assembly of magnetic nanoparticles in aqueous solution leads to a change in the proton nuclear spin-spin relaxation time (T<sub>2</sub>) of water molecules.<sup>4</sup> Consequently, when used as probes for dynamic biological phenomena,  $\Delta T_2$  values serve as indicators of not only the switching of magnetic nanoparticles between dispersed and assembled states, but also the degree of assembly of target molecules. In order to maximize MRI signal enhancing effects, nanoparticles with strong magnetism are desirable.<sup>5</sup> Although ferromagnetic metals, such as Fe, Co, and Ni, possess stronger magnetic moments than that of currently widely used iron oxide nanoparticles, they cannot be directly used for biological applications due to their high reactivity in aqueous environments.<sup>6</sup> In this study, we have developed highly stable metal-based heterodimer nanoparticles, which have both large magnetic moments and high colloidal stability. We have used them as probes for direct MRI observation of A $\beta$  assemblies at early stages of the progressive assembly.

The heterodimer nanoparticles, consisting of a Co magnetic core and a Pt shell that is directly fused to an Au nanoparticle (Fig. 1(a)), are prepared from Co@Pt nanoparticles.<sup>7</sup> Au is

then deposited on the Co@Pt nanoparticles via hydrogenation of AuCl(PPh<sub>3</sub>) by purging with a 4% H<sub>2</sub>/Ar mixture.<sup>8</sup> The Co@Pt-Au nanoparticles obtained in this manner have a heterodimer structure of roughly 15 nm, composed of 6 nm Co@Pt and 9 nm Au (Fig. 1(b)). The measured face-centered cubic (111) lattice distances of the Pt shell and Au deposition are 2.2 and 2.4 Å respectively, which are consistent with known values<sup>9</sup> (Fig. 1(d)). From energy dispersive X-ray spectroscopic (EDS) analysis, compositional changes corresponding to the Pt (shell), Co (core) and Au clearly confirm the formation of Co@Pt-Au nanoparticles (Fig. 1(d)). Co@Pt and Co@Pt-Au nanoparticles possess almost identical

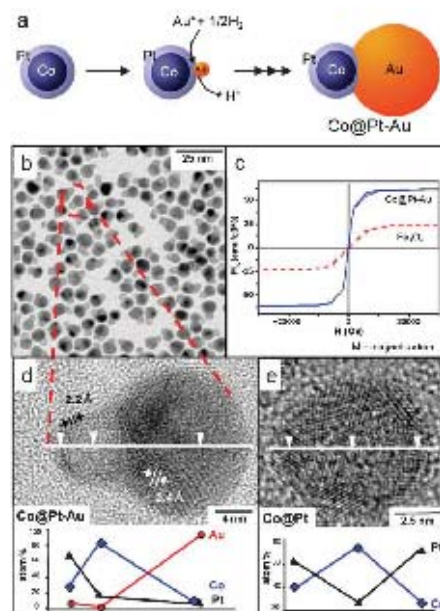


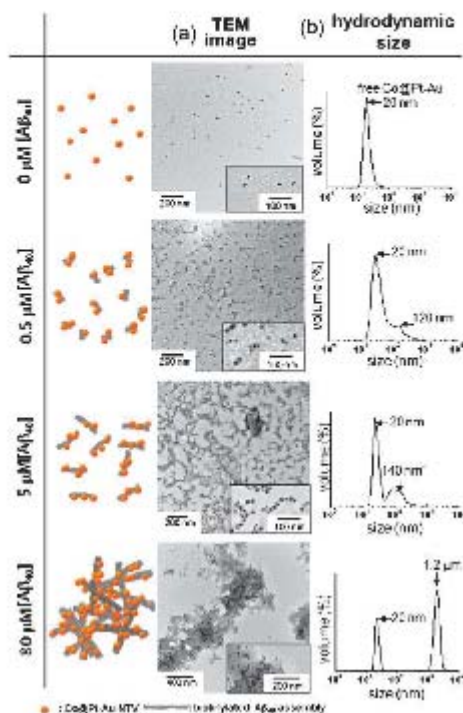
Fig. 1 (a) Schematic of Co@Pt-Au nanoparticle synthesis. (b) Transmission electron microscopy (TEM) image of Co@Pt-Au. (c) Comparison of magnetization per mass of magnetic component of Co@Pt-Au (Co: 4 nm; blue solid line) with 4 nm-sized iron oxide (red dashed line). High resolution TEM (HRTEM) images and composition analysis of (d) Co@Pt-Au and (e) Co@Pt at different spot positions of nanoparticles using EDS (Co: ♦, Pt: ▲, Au: ●).

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† Electronic supplementary information (ESI) available: Experimental details on the fabrication of Co@Pt-Au, conjugation of Co@Pt-Au with NTV, preparation of A $\beta$  solutions and disassembly of A $\beta$  under diluted conditions. See DOI: 10.1039/b803294g





**Fig. 3** Co@Pt-Au-NTV nanoparticle aggregates induced by Aβ<sub>40</sub> assemblies. (a) TEM and magnified TEM images (inset) show progressive structural changes of nanoparticle assemblies, depending on Aβ<sub>40</sub> concentration (0, 0.5, 5 and 80 μM). (b) DLS data also show the size changes of Co@Pt-Au-NTV-Aβ<sub>40</sub> assemblies as the Aβ<sub>40</sub> concentration increases.

changes taking place at the early stages of assemblies including protofibrils. On the other hand, at Aβ<sub>40</sub> concentrations over 10 μM, the MRI response to the Aβ<sub>40</sub> process is not as large.

Early stage protofibrils, with strong T<sub>2</sub> changes, are in a reversible state. For example, protofibrils that are produced at Aβ<sub>40</sub> concentrations around 5 μM reversibly disassemble. Only dispersed Co@Pt-Au (hydrodynamic size of ~20 nm) is observed when the protofibrils are diluted to 0.1 μM (Aβ<sub>40</sub>) and kept for 4 d (ESI†). In contrast, fibrils generated at 80 μM (Aβ<sub>40</sub>) maintain their micron-sized irregular aggregate form without a significant change in their size when diluted. Since it is possible to reverse and retard the Aβ assembly process, the ability to detect Aβ assemblies in their reversible stages is important in the context of Alzheimer's disease treatment.<sup>13</sup>

In summary, we have demonstrated that Co@Pt-Au nanoparticles that have enhanced magnetic properties and high colloidal stability can be used in conjunction with MRI to monitor key structural stages of Aβ assemblies. The exceptional, dramatic changes that take place in MR signals during Aβ assembly enable the detection of Aβ protofibrils in the early reversible stages. This is an important finding because information of this type could be

used in a therapeutic approach to Alzheimer's disease that involves the reversible disassembly of Aβ protofibrils. Furthermore, the magnetic nanoparticle-assisted MRI detection system could be applied as a sensitive probe of protein self-assemblies including prion, α-synuclein and Huntingtin.

We thank Dr Y.-w. Jun for his helpful discussions and M.-y. Kim for her graphics work. This work is supported in part by NRL (R0A-2006-000-10255), NCI Center for Cancer Nanotechnology Excellence, **AOARD (FA4869-07-1-4016)**, NCRC (R15-2004-024-00000-0), Nano/Bio Science & Technology Program (M10503000218-05M0300-21810), the KRC of Fundamental Science & Technology (2N30630-07-259), the BK 21 Project and Seoul Science Fellowship.

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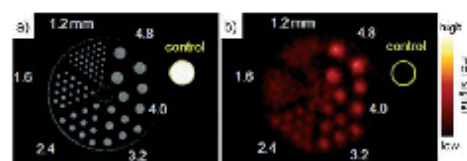
# A Hybrid Nanoparticle Probe for Dual-Modality Positron Emission Tomography and Magnetic Resonance Imaging\*\*

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As the accurate imaging of biological targets becomes an increasingly important tool for understanding basic biological phenomena and for fault-free diagnosis of various diseases,<sup>[1]</sup> current single-modality imaging methods tend to be inadequate. Therefore, multimodal imaging can be an essential tool in state-of-the-art imaging research and also standard practice in the clinic.<sup>[2]</sup> By combining dual- and triple-modality methods, many shortcomings that are present for single-modality imaging methods can be overcome. For example, the simultaneous use of positron emission tomography (PET), for its highly sensitive functional imaging, and computed tomography (CT), for its ability to provide clear anatomical information, has been demonstrated for the early detection of cancer.<sup>[2b]</sup> Several combinations of imaging methods with different modalities, such as magnetic resonance (MR)/optical<sup>[2d]</sup> and PET/near-infrared optical fluorescence (NIRF),<sup>[2d]</sup> are possible. Recently, the development of a dual-modality diagnostic instrument for PET/MR imaging (MRI) has been demonstrated.<sup>[2d]</sup>

In addition to the instrumental advances of the diagnostic hardware, the development of nanoparticle-based probes is a prerequisite for successful multimodal diagnostics.<sup>[3]</sup> Quantum dots have been labeled with radioisotopes or paramagnetic metal ions to serve as NIRF-based dual-modality probes.<sup>[3a–d]</sup> Superparamagnetic iron oxide (SPIO) nanoparticles have been labeled with fluorescent dyes for MR/optical dual-modality imaging.<sup>[3b]</sup> The SPIO nanoparticle conjugated with <sup>64</sup>Cu and fluorescent dye as a new class of trimodal probe was demonstrated for macrophage detection in atherosclerotic plaques by using PET/CT imaging in comparison to MRI.<sup>[3b]</sup>

Herein, we report a magnetic-nanoparticle-based PET/MRI probe and the evaluation of its effectiveness as an in vivo dual-modality imaging agent. Noninvasive imaging and three-dimensional tomography are important advantages of PET and MRI.<sup>[1]</sup> In Figure 1, the imaging characteristics of MR and



**Figure 1.** Comparison of spatial resolution of a) MRI and b) PET techniques with a Derenzo phantom (circle diameters of 1.2, 1.6, 2.4, 3.2, 4.0, and 4.8 mm), which contains the contrast agents MnMEIO (50  $\mu\text{g mL}^{-1}$  (Mn + Fe) and <sup>125</sup>I (2.5  $\mu\text{Ci mL}^{-1}$ ); yellow circle: water (control). MnMEIO = Mn-doped magnetism-engineered iron oxide.

PET are compared by using a Derenzo phantom filled with a solution of contrast agents. In the MR image, all circles ranging from 1.2 to 4.8 mm in diameter are well resolved (Figure 1a) and it is possible to go further down to a resolution of 250  $\mu\text{m}$  (see the Supporting Information, Figure S1). On the other hand, the PET image shows relatively poor spatial resolution and only circles larger than 2.4 mm in diameter are resolved (Figure 1b).

Although the MR probe provides better spatial resolution, in terms of sensitivity the PET probe shows an enhanced contrast effect, with picogram-scale sensitivity compared to the microgram-scale sensitivity of the MR probe (see below).<sup>[1a]</sup> Therefore, combining the merits of the individual imaging techniques through a single hybrid probe would make PET/MR dual modality an ideal candidate for a variety of biological studies. We chose the sentinel lymph node (SLN) as a model biological subject. The lymphatic system is an

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[\*\*] This work was partially supported by the KRF (MOEHRD, Basic Research Promotion Fund) (KRF-2007-331-E00166), KOSEF (R15-2004-033-06001-0), NRL (R0A-2006-000-10255), AOARD (FA4869-07-1-4016), Nano/Bio Program (M10503000218-05M0300-21810), the Korea Research Council of Fundamental Science & Technology, NCI Center for Cancer Nanotechnology Excellence, and the BK21 program of chemistry and medical college. Production of <sup>125</sup>I was supported partially by the QURI project of MOST and KOSEF.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.200801369>.